Standard Operating Procedure

TITLE: Aqueous Sample Preparation for the Analysis of Polyaromatic Hydrocarbons - HPLC and GC/MS

DEPARTMENT Semivolatile Organics

REFERENCES: SW-846 3500B Organic Extraction and Preparation
Revision 2, December 1996
SW-846 3510C Separatory Funnel Liquid-Liquid Extraction
Revision 3,December 1996

PROCEDURE SUMMARY:

Waters are collected in a teflon lined cap amber bottle and must be extracted within 7 days of sample collection. All samples must be stored on ice or between 0.0-4.0 C prior to extraction. A known amount of sample is introduced into a separatory funnel with a solvent. Diluent solvent is collected and blown down to a final volume. The final extract volume is stored in a crimp top auto sampler vial from which an aliquot is injected onto the HPLC or GC/MS system, depending on the determinitive method to be used.

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1.0 SCOPE AND APPLICATION

1.1 This method describes a procedure for isolating organic compounds from aqueous samples. The method also describes concentration techniques suitable for preparing the extract.

1.2 This method is applicable to the isolation and concentration of water insoluble and slightly water-soluble organics in preparation for a variety of chromatographic procedures.

2.0 SUMMARY OF METHOD

2.1 A measured volume of sample, usually 1 liter, at a neutral pH, is serially extracted with methylen chloride using a separatory funnel. The extract is dried, concentrated, and, as necessary, exchanged into a solvent compatible with the cleanup or determinative method to be used.

3.0 INTERFERENCES

3.1 Solvents, reagents and glassware can interfere with sample analysis. En Chem purchases high quality solvents and reagents to eliminate this potential of contaminations and require these vendors to stand behind claims made on the solvents purchased. En Chem has adopted and implemented SOPs addressing proper glassware cleaning techniques, tailored to the department using the apparatus.

4.0 APPARATUS AND MATERIALS

4.1 Labline Automated Shaker System, including 2-liter extraction glassware with Teflon stopcock.

4.2 Drying funnel - glass funnel with glass wool at the bottom and filled with baked sodium sulfate.

4.3 Zymark 1.0 ml endpoint tubes.

4.4 Zymark TurboVap II Concentration Workstation.

4.5 Glass beakers-six 250 mL.

4.6 Vials - 2 mL, glass, with PTFE/silicone rubber liner crimp tops.

4.7 pH indicator paper - pH of sample indicated by color coding.

4.8 Disposable pasteur pipettes with pipette bulbs.

4.9 Syringes - 50 uL and 1.0 mL. for HPLC; 10 uLand 100uL for GC/MS.

4.10 Graduated cylinder - 1 liter.

4.11 High purity nitrogen gas source.

4.12 Aluminum foil for HPLC.

4.13 Volumetric Flasks- 5.0 mL and 50.0mL for HPLC; 25.0mL and 50.0mL for GC/MS.

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. Reagents should be stored in glass to prevent the leaching of contaminants from plastic containers.

5.2 Organic-free reagent water - Culligan purified/treated water.

5.3 Sodium sulfate (granular, anhydrous), Na2SO4. Purify by heating at 400-C for 4 hours in a shallow tray, or by precleaning the sodium sulfate with methylene chloride. If the sodium sulfate is precleaned with methylene chloride, a method blank must be analyzed, demonstrating that there is no interference from the sodium sulfate. J.T. Baker(ACS).

5.4 Extraction/exchange solvents.

5.4.1 Methylene chloride, CH2Cl2 - Pesticide quality or equivalent - Fisher.

5.4.2 Acetonitrile, CH3CN - Pesticide quality or equivalent - Burdick & Jackson HPLC grade.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 Waters should be contained in unpreserved liter amber bottles. All samples should be transported on ice and stored at a temperature of 4C. Extraction must take place within 7 days of sampling. Analysis must take place within 40 days of extraction.

7.0 PROCEDURE

7.1 Decant the liquid sample (1.0 L) into the extraction glassware. If less than 1.0 L is present, mark the miniscus of the sample with a marker, and save the amber jar for measurement with a 1.0-L graduated cylinder. If high concentrations are anticipated, a smaller volume may be used and then diluted with organic-free reagent water to 1 liter.

Note: All glassware and other materials that come into contact with the sample, directly or indirectly, are first rinsed with the the extraction solvent.

7.2 Spiking/Surrogate solutions:HPLC

7.2.1 Spike - Dilute 50 uL of the stock standard, Accustandard M-610-QC-FL-R1 variable concentration, to 5.0 mL with methylene chloride. Spike 1.0 mL into BS, BSD, MS and MSD.

7.2.2 Surrogate - Dilute 50 uL of the stock standard surrogate, Chem service F1028S 9,10- DIphenyl anthracene at 100 ppm, to 50 mLs in a volumetric flask. Spike 1.0 mL into all QC and samples.

7.3 Spiking/Surrogate solutions: GC/MS

7.3.1 Spike-Dilute 10 uL of stock standard, Accustandard M-610-FL-R-5X at 500ppm, to 25.0 mL with methylene chloride. Spike 1.0 mL into BS, BSD, MS and MSD.

7.3.2 Surrogate-An intermediate stock solution is prepared by diluting 300 uL of Restek B/N mix 31082 at 5000 ppm, to 10.0 mL with methylene chloride to make a 150 ppm B/N intermediate solution. The intermediate is further diluted to prepare a daily working solution by diluting 66.66 uL of the 150 ppm B/N intermediate solution to 50.0mL with methylene chloride. Spike 1.0 mL to all QC and samples.

7.4 Add 60 mL of methylene chloride to the separatory funnel.

7.5 Seal the extraction glassware and set the shaking time to 2.0 minutes. Set the speed to 30 turns/minute full speed).

NOTE: Methylene chloride creates excessive pressure very rapidly. However, venting takes place through a small hole in the side cap of the extraction glassware. For this reason, the automated shaker should always be placed in a hood-ventilation system when using solvent.

7.6 Allow the organic layer to separate from the water phase for a minimum of 2 minutes. If the emulsion interface between layers is more than one-third the size of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample and may include stirring, filtration of th emulsion through glass wool, centrifugation, collection of the emulsion in a beaker of sodium sulfate and stirring to break the emulsion, or other physical methods. Collect the solvent extract in 200ml TurboVap tubes. (The tubes should have drying funnels, which contain glass wool and sodium sulfate, positioned on top of the mouth of the tube, for filtration.)

7.7 Repeat the extraction two more times using 40-mL portions of fresh solvent (Sections 7.4 through 7.5). combine the three solvent extracts together in the concentrator tubes. Label the combined extract with its appropriate sample number.

7.8 Perform the concentration using the Zymark evaporative concentrator technique (Sections 7.9.1 through 7.9.5).

7.9 Zymark TurboVap II Concentration Technique

7.9.1 Rinse the drying funnels with small amounts - 20 mLs of methylene chloride. These rinses can be filtered into the concentrator tubes.

7.9.2 Cover the mouth of the concentrator tubes with aluminum foil if the sample is to be analysed by HPLC. Foil is not used if the analysis is to be done by GC/MS.

7.9.3 Place the concentrator tubes in the TurboVap II water bath, which is set at 50 C for HPLC analysis and at 35 C for GC/MS analysis. Set the TurboVap in sensor mode. Start each cell that contains a concentrator tube and set the pressure on the TurboVap to 11 psi.

7.9.4 When the extract is concentrated to 0.75 ml, the nitrogen will automatically shut off and an alarm will sound. At this time, add approximately 8.0 ml of acetonitrile for a solvent exchange if the sample is to be analysed by HPLC (then proceed to step 7.9.5). For GC/MS analysis, solvent exchange is not performed. The concentrator tube is removed from the turbovap and allowed to cool. Once cool the solvent in the concentrator tube is used to rinse down the conical portion of the tube. Additional methylene chloride is added to bring the final volume to 1.0 mL (then proceed to step 7.9.6).

7.9.5 Concentrate the extract to 0.75 ml using the sensor mode. Add about 8.0 ml of acetronitrile again and concentrate to 0.75 ml. Remove the tube from the water bath and allow to cool. Rinse only the conical part of the tube, bringing the final volume of the extract to 1.0 ml.

7.9.6 Tranfer the extract to 2.0 ml crimp vials.

7.9.7 The samples are now ready for HPLC or GC/MS analysis.

8.0 Deviations from the cited references

8.1 Method 3510C recommends the use of Kaderna -Danish glassware and a water bath for the concentration step. Here the use of the Turbo-vap system by Zymark is used to accomplish this portion of the method.